

Claims

1. A method of making a replicatable RNA template having a selected affinity to a target, comprising the steps of:
 - 5 a.) applying a selection to a first generation comprising at least one replicatable RNA template as said replicatable RNA template is replicated by a RNA polymerase to form at least one subsequent generation comprising a replicatable RNA template, said selection based on the affinity of said replicatable RNA template of different generations to said target, said RNA polymerase introducing genetic variability between generations of said replicatable RNA template to produce replicatable RNA templates having different
10 affinities to said target; and
 - b.) separating the replicatable RNA templates on the basis of the affinity of said replicatable RNA template to the target.
- 15 2. The method of claim 1 wherein said selection is applied by passing a solution comprising said first and subsequent generations of said replicatable RNA template through or over a fixed medium, said fixed medium having said target immobilized, such that replicatable RNA templates having low affinity for the target and replicatable RNA templates having high affinity for the target assume different positions in said solution as
20 said solution passes through said fixed medium.
3. The method of claim 2 wherein said fixed medium is provided with reagents and an RNA polymerase.
- 25 4. The method of claim 3 wherein said RNA polymerase is selected from the group of polymerases consisting of Q-Beta replicase, MS2 replicase, GA replicase, and SP replicase and modifications thereof.
5. The method of claim 4 wherein said RNA polymerase is non-selective with
30 respect to template.

6. The method of claim 1 wherein said selection is applied by placing said first generation and target in a solution, and after at least one subsequent generation has been formed, forming a first fraction of said solution and a second fraction of said solution, said first fraction substantially devoid of target, said solution of said first fraction
5 containing replicatable RNA templates having lower affinity than replicatable RNA templates in said second fraction, said second fraction having said target containing replicatable RNA templates having higher affinity than replicatable RNA templates in said first fraction.
- 10 7. The method of claim 6 wherein said separation is performed by one or more of the processes selected from the group consisting of centrifugation, filtration, electric field electrophoresis, and precipitation.
8. The method of claim 6 wherein at least one fraction is provided with additional
15 reagents and RNA polymerase in the presence of target.
9. The method of claim 6 wherein said selection is applied in a plurality of vessels containing said solution to reduce selection based on the composition of the vessel.
- 20 10. The method of claim 1 wherein said selection is applied by an enzyme having a catalytic entity and a target entity.
11. The method of claim 10 wherein said catalytic entity is derived from one of the RNA dependent RNA polymerases selected from the group consisting of Q-Beta
25 replicase, MS2 replicase, GA replicase, and SP replicase and modifications thereof.
12. The method of claim 10 wherein said target is incorporated into one or more subunits of the polymerase.
- 30 13. The method of claim 10 wherein said target is covalently bonded to said catalytic entity.

14. A device for making a replicatable RNA template having a selected affinity to a target, comprising:

a.) means for applying a selection to a first generation comprising at least one replicatable RNA template as said replicatable RNA template is replicated by a RNA polymerase to form at least one subsequent generation comprising a replicatable RNA template, said selection based on the affinity of said replicatable RNA template of different generations to said target, said RNA polymerase introducing genetic variability between generations of said replicatable RNA template to produce replicatable RNA templates having different affinities to said target; and

b.) means for separating the replicatable RNA templates on the basis of the affinity of said replicatable RNA template to the target.

15. The device of claim 14 wherein said means for applying a selection is a fixed medium said selection applied by passing a solution comprising said first and subsequent generations of said replicatable RNA template through said fixed medium, said fixed medium having said target immobilized, such that replicatable RNA templates having low affinity for the target and replicatable RNA templates having high affinity for the target assume different positions in said solution as said solution passes through said fixed medium.

16. The device of claim 11 wherein said fixed medium is held in a column.

17. A kit for making a replicatable RNA template having a selected affinity to a target, comprising:

a.) means for applying a selection to a first generation comprising at least one replicatable RNA template as said replicatable RNA template is replicated by a RNA polymerase to form at least one subsequent generation comprising a replicatable RNA template, said selection based on the affinity of said replicatable RNA template of different generations to said target, said RNA polymerase introducing genetic variability between generations

of said replicatable RNA template to produce replicatable RNA templates having different affinities to said target; and

b.) means for separating the replicatable RNA templates on the basis of the affinity of said replicatable RNA template to the target.

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18. The Kit of claim 17 wherein said RNA polymerase is *evolvase*.